

AONSA Young Research Fellow Final Report

Name : Dr Indri Badria Adilina
Affiliation : National Research and Innovation Agency (BRIN)
Facility : Australian Center for Neutron Scattering (ANSTO)
Supervisor : Dr Kathleen Wood
Program : 1/10/2022 – 30/09/2023

Research Title:

Neutron scattering studies of the nanoparticle-protein interaction on modified geothermal silica

Research Summary:

Mesoporous silica nanoparticles (MS) are widely used in catalysis, drug delivery, biological imaging and biosensors, and their interaction with biomolecules has been the focus of intensive research since they combine a high inner surface area with pore sizes sufficient to host most proteins and enzymes. Structural changes in these systems, however, could lead to protein misfolding and aggregation, which have been correlated with a loss of functionality. For this reason, a detailed understanding of the bio-nano interface is essential to control the biological response and stability required for their applications. In this context, we have synthesised four types of MS from geothermal waste as the silica source using a cetyltrimethylammonium bromide (CTAB) template and water or water-alcohol (ethanol, propanol, or butanol) mixtures as the solvent (GMS, GMS-Et, GMS-Pr, GMS-Bu). GMS shows significant increase in the surface area as compared to geothermal Si from 37 up to 1177 m²g⁻¹, depending on the solvent used during synthesis. SANS and USANS data show that GMS particles are polydisperse with calculated particle size diameter from 200 nm to more than 20 μ m. SAXS, WAXS, TEM and BET data confirms that a hexagonal ordered pore structure is present for all the GMS except for GMS-Bu with pore size between 2.97-3.78 nm. Adsorption studies of lysozyme (LYS) into MS was carried out at various pH of 4, 7 and 9. LYS was adsorbed higher at pH 9 for both the ordered and non-ordered GMS (216 to 387 mg/g Si). SANS, USANS and QENS data reveal the strong interaction of LYS with MS resulting in slower dynamics after the immobilisation. Adsorption of bovine serum albumin (BSA) was also performed with ongoing data interpretation.

Research Activities:

During the AONSA YRF, Indri has performed the following activities:

- Submitted a proposal related to her AONSA YRF work through the ANSTO beamtime proposal system which was peer-reviewed and awarded beamtime.
- Become an independent operator of three neutron scattering instruments: Kookaburra, (ultra small angle scattering), Quokka (monochromatic small angle neutron scattering) and Emu (backscattering spectroscopy).
- Produced multi-component samples of mesoporous silica and proteins. The sample conditions, including pH, have then been optimised for use in neutron scattering and characterised with basic lab techniques.
- Performed experiments characterising the solution state structures of the multi-component samples on Quokka and Kookaburra.
- Performed experiments characterising the dynamics as a function of temperature of the multi-component samples on Emu.
- Use her connections to BRIN and Synchrotron Light Research Institute (SLRI) to access instruments for BET surface area, TEM, SAXS, and WAXS, which supports the findings of her neutron scattering research at ANSTO.
- Created and submitted a new proposal related to biochar materials through the ANSTO beamtime proposal system which has been awarded beamtime on Kookaburra, Quokka, and Taipan (inelastic neutrons scattering).

- Presented her work at the International Conference on Crystallography (IUCr) in Melbourne, the Gordon Research Conference in Ventura USA, and at the ANSTO user meeting in Sydney. She received a grant from the organisers to cover attending the conferences.
- Organised a virtual workshop with the Indonesian neutron scattering community, where Indri showcased Australian neutron scattering expertise and her AONSA YRF work.
- Submitted a funding application to continue collaboration with ANSTO – APEC Australia Women in Research Fellowship and KONEKSI project.
- Extend her time at ANSTO for 6 months and will be working on the analysis and interpretation of her Emu, Quokka and Kookaburra data and drafting a joint publication.

Experimental Results

SANS and USANS

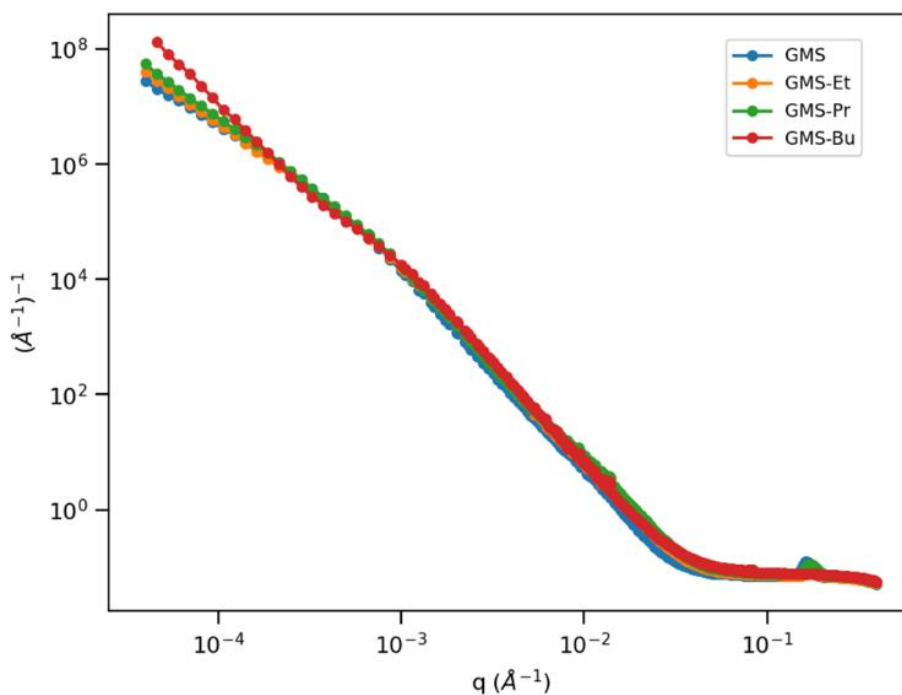


Figure 1. SANS and USANS analysis of GMS materials

- Water or water-alcohol (ethanol, propanol, or butanol) mixtures as the synthesis solvent gives different effects on the structure
- Alcohol induces structural transformations of CTAB aggregates and effects the ordered pore structure formed
- SANS and USANS data shows that GMS particles are polydisperse with calculated particle size diameter from 200 nm to more than 20 μm

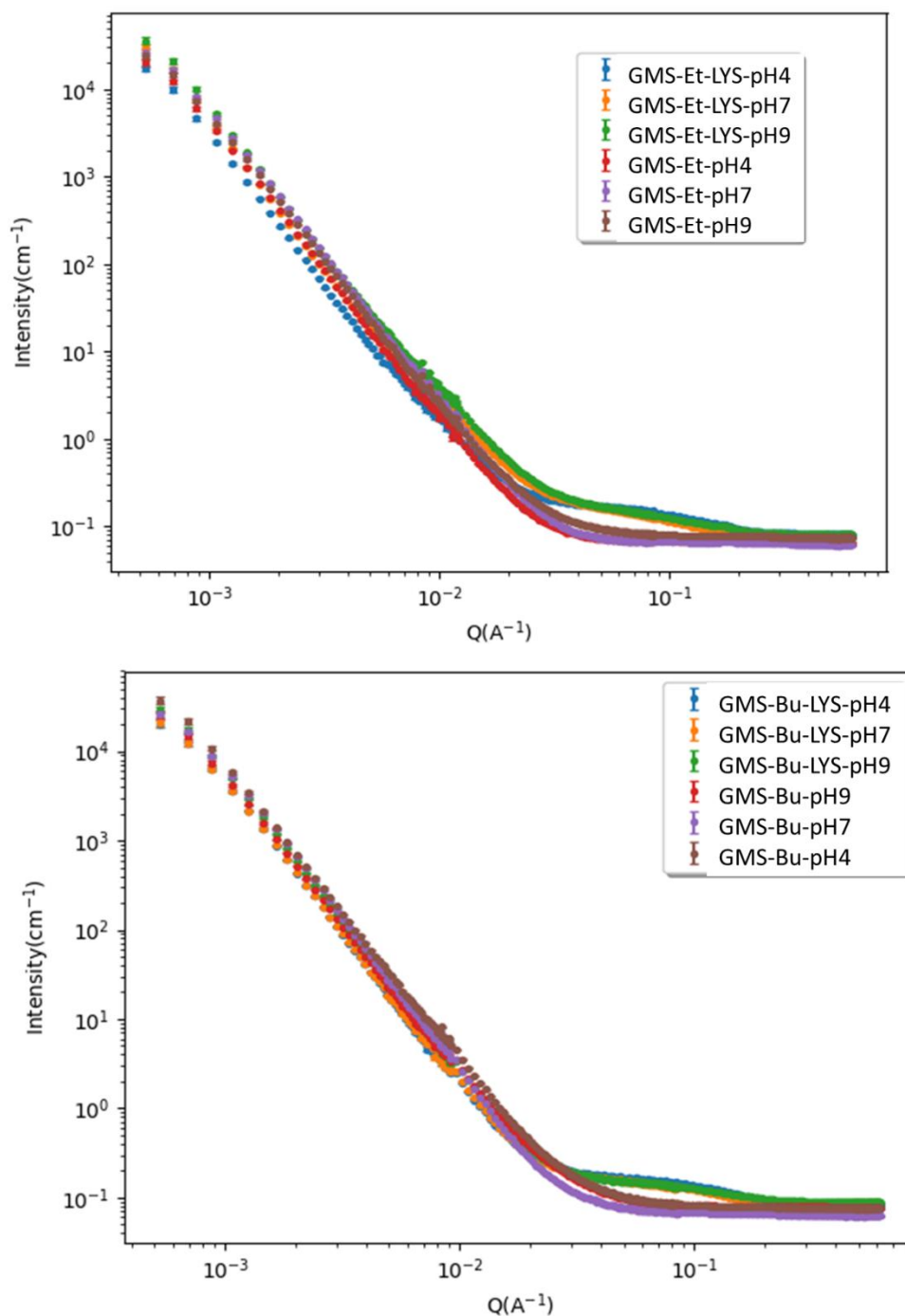


Figure 2. SANS and USANS analysis of GMS materials before and after adsorption of protein (LYS) at different pH: Ordered structure (top) and non-ordered structure (bottom)

- The build-up of scattering intensity around $Q = 10^{-1}$ Å suggests particle aggregation due to strong interaction of LYS with silica
- The strong interaction was observed for both the ordered (GMS-Et-LYS) and non-ordered mesoporous silica (GMS-Bu-LYS)
- Further fitting of the SANS data is essential to provide the details of the silica-protein interaction

QENS

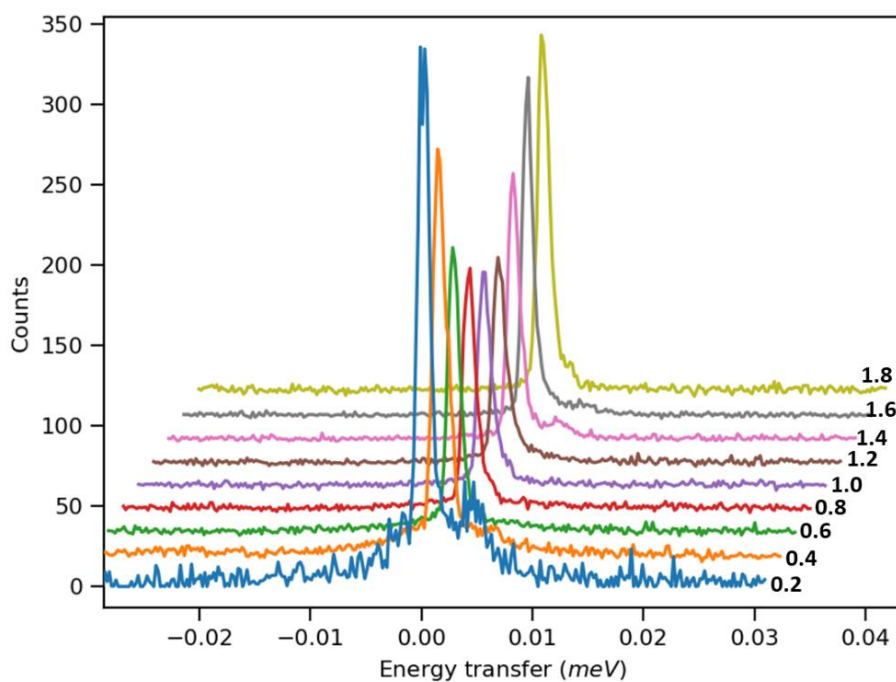
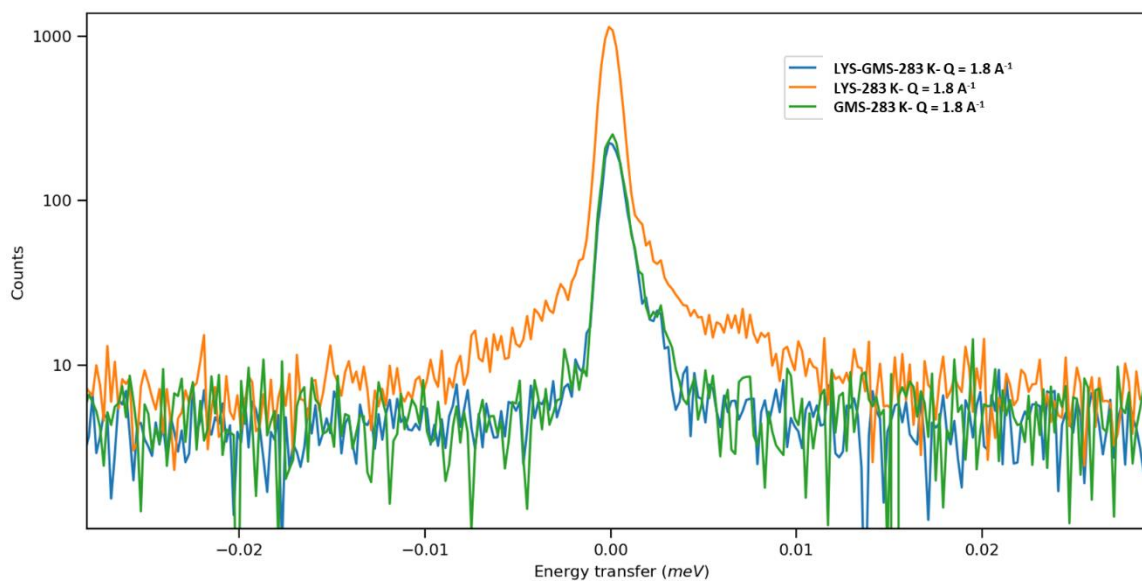


Figure 3. QENS analysis of GMS material before and after adsorption of protein (LYS) at 283 K at $Q = 1.8 \text{ \AA}^{-1}$ (top) and various Q (bottom)

- QENS spectra at 283 K and $Q = 1.8 \text{ \AA}^{-1}$ show that the dynamics of LYS is slower after immobilisation on silica, demonstrated by narrower QENS peak width
- Strong interaction of protein with silica resulting in slower dynamics was observed after immobilisation
- Further fitting of the QENS data as a function of temperature is essential to provide the details of the dynamics in the silica-protein system



Neutron scattering studies of mesoporous silica synthesised from a geothermal source and their protein adsorption studies

Indri Adilina^{1,3}, Arum Patriati², Alice Klapproth³, Liliana de Campo³, Kathleen Wood³

¹Research Center for Chemistry, National Research and Innovation Agency, Tangerang Selatan, Indonesia 15314

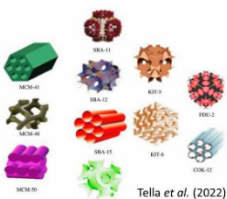
²Research Center for Radiation Detection and Nuclear Analysis, National Research and Innovation Agency, Tangerang Selatan, Indonesia 15314

³Australian Centre for Neutron Scattering, Australian Nuclear Science and Technology Organization, New Illawarra Road, Lucas Heights, NSW 2234, Australia

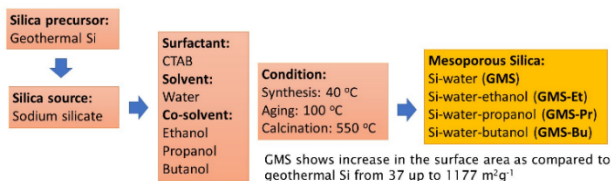
Email: indro30@brin.go.id, adilinai@ansto.gov.au

Introduction

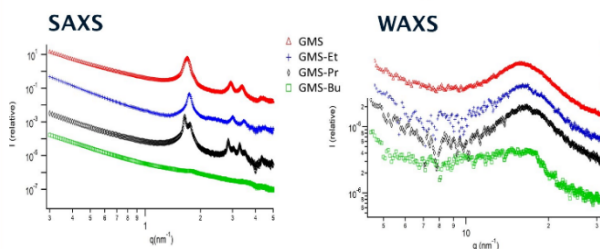
- Mesoporous silica is widely used in catalysis, drug delivery, biological imaging and biosensors
- Their interaction with biomolecules is important as they combine a high inner surface area with pore sizes sufficient to host most proteins and enzymes
- Indonesia has the world's largest geothermal silica source



Synthesis of Mesoporous Silica



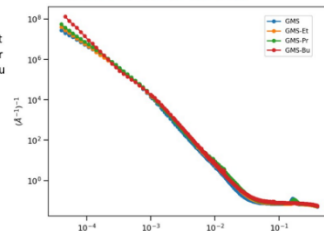
Ordered Structure of Mesoporous Silica



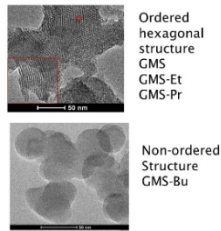
No	Sample	1 st peak SAXS q/nm ⁻¹	1 st peak SAXS d/nm	2 nd peak SAXS q/nm ⁻¹	2 nd peak SAXS d/nm	3 rd peak SAXS q/nm ⁻¹	3 rd peak SAXS d/nm	WAXS q/nm ⁻¹	WAXS d/nm
1	GMS	1.69	3.72	2.93	2.15	3.23	1.95	16.02	0.39
2	GMS-Et	1.74	3.62	3.01	2.09	3.46	1.82	14.95	0.42
3	GMS-Pr	1.65	3.82	2.88	2.18	3.27	1.92	16.58	0.37
4	GMS-Bu	1.75	3.58	3.03	2.07	3.50	1.79	-	-

The first peak in the SAXS scattering profile that is an inter-correlation peak which indicates the distance between the pores or d-spacing

USANS and SANS

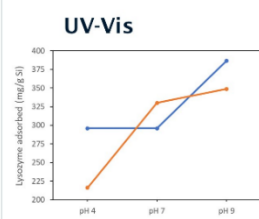


TEM



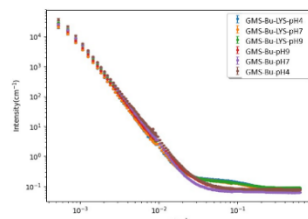
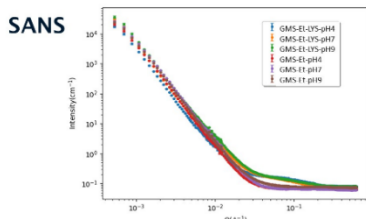
- Water or water-alcohol (ethanol, propanol, or butanol) mixtures as the synthesis solvent gives different effects on the structure
- SAXS, WAXS and TEM data confirms that a hexagonal ordered pore structure is present for all the GMS except for GMS-Bu with pore size between 2.97-3.78 nm
- USANS and SANS data shows that GMS particles are polydisperse with calculated particle size diameter from 200 nm to more than 20 um

Protein Adsorption Studies

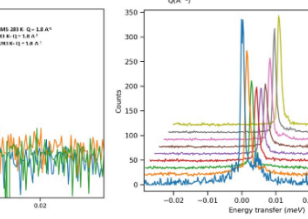
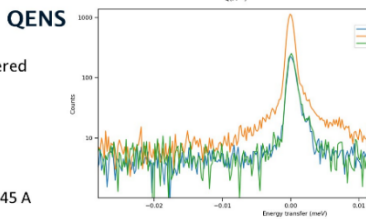


Lysozyme (LYS) was adsorbed higher at pH 9 for both the ordered and non-ordered GMS (216 to 387 mg/g Si)

Properties of LYS
Molecular mass : 14400 kDa
Isoelectric point : 11
Dimension : 30 Å × 30 Å × 45 Å



The build-up of scattering intensity around $Q = 10^{-1}$ Å suggests particle aggregation due to strong interaction of LYS with silica



Quasielastic neutron scattering (QENS) spectra at 283 K and $Q = 1.8$ Å⁻¹ show that the dynamics of LYS is slower after immobilisation on silica, demonstrated by narrower QENS peak width

Conclusion

- Mesoporous silica synthesised from a geothermal source using CTAB as surfactant and water or water-ethanol and water-propanol as the solvent gives MCM-41 like ordered hexagonal structure
- Strong interaction of protein with silica resulting in slower dynamics was observed after immobilisation
- Further fitting of the SANS and QENS data is essential to provide the details of the interaction and dynamics in the silica-protein system

Acknowledgements

The AONSA Young Research Fellowship is thanked for the research grant and travel support. We also thank the Indonesian Neutron Scattering Society and Synchrotron Light Research Institute for their support

